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Effects of Culture in High CO_2 on the Photosynthetic Physiology of *Fucus serratus*

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The lower intertidal macroalga *Fucus serratus* was cultured in high CO_2 (5 kPa CO_2) and air (33 Pa CO_2) for three weeks to investigate possible adjustments to its photosynthetic physiology. When cultured in high CO_2, the CO_2 compensation point increased and affinity for CO_2 decreased when measured with the thallus exposed to air. For the submersed alga, the pH compensation point and photon yield decreased. There was little change in the level of chlorophyll *a* and the rate of dark ^{14}C fixation following culture at high CO_2. The shift from a C_4-type to C_3-type gas exchange physiology is comparable with the response of microphytes to high CO_2 acclimation. At the end of the three week period, the high CO_2 algae still possessed the ability to utilize bicarbonate ions as a source of inorganic carbon, but with a reduced capacity. As there was no change in the rates of dark fixation between low and high CO_2 cultured algae, it is concluded that this process is not directly involved in the inorganic carbon concentrating mechanism.

The photosynthetic characteristics of microalgae and cyanobacteria can be controlled by growing the cells under high CO_2 partial pressure (1–5 kPa CO_2) or under air-equilibrium [33 Pa CO_2 (Berry *et al.*, 1976)]. Cells that are adapted to a low CO_2 environment typically have high apparent affinities for CO_2, the ability to accumulate inorganic carbon so that the internal concentration is greater than the external concentration, a low CO_2 compensation point, a high pH compensation point, reduced photorespiratory activity, and show a low discrimination between ^{12}C and ^{13}C (see Raven, 1985). High/low CO_2 acclimation phenomena are known for a number of freshwater macrophytes, both algal (*Chara corallina*; Lucas & Brechignac, 1987) and vascular (the submerged *Elodea canadensis* Michx.; Elzenga, Prins & Kuiper, 1987; and the floating *Eichhornia crassipes* [Mart.] Solms.; Larigauderie, Roy & Berger, 1986; Spencer & Bowes, 1986). The limited work on marine macroalgae suggests that these algae have the ability to utilize the bicarbonate ions as a source of inorganic carbon (Johnston & Raven, 1986*a*; Cook, Lanaras & Colman, 1986), and exhibit reduced photorespiration (Bidwell & McLachlan, 1985; Johnston & Raven, 1987). Macroalgae have been described as possessing photosynthetic characteristics which are "C_4-like" because the physiology is similar to C_4 higher plants although the biochemistry of most investigated species is C_3 with PGA being the first acid stable produce of $^{14}CO_2$ fixation (Kerby & Raven, 1985; Johnston & Raven, 1987). Intertidal macroalgae experience a constant emersion/immersion cycle and they have the ability to assimilate inorganic carbon in both air and water (Johnston *et al.*, 1974; Johnston & Raven, 1986*b*; Surif & Raven, 1989).

In our laboratory the mechanism behind the difference between the photosynthetic biochemistry and physiology of macroalgae is being investigated via three lines of research: the role that inhibitors of carbonic anhydrase have on the gas exchange characteristics (Bidwell & McLachlan, 1985; Reiskind, Seamon & Bowes, 1988; Smith & Bidwell, 1987, 1989); direct measurement of internal dissolved inorganic carbon (Smith & Bidwell, 1989); and the role of β-carboxyla-

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tion catalysed by phosphoenolpyruvate carboxykinase [PEPCK (Bowes, 1985; Reiskind, Seamon & Bowes, 1988; Johnston & Raven, 1989)]. In this paper we describe some effects of culturing *Fucus serratus* L. in high and low CO_2 environments.

MATERIALS AND METHODS

Young, 30 mm long plants of *Fucus serratus* L. were collected in mid November 1988 from the low tide mark at Arbroath, Scotland (O.S. Ref. NO 659 412). On returning to the laboratory the material was washed in filtered seawater and examined; plants which had epiphytes or diseased tissue were discarded. Fifteen plants were cultured for three weeks in 2·5 litres of enriched seawater as described by Davison & Davison (1987) (cf. Provasoli, 1968); the culture medium was changed every 2 to 3 days. The flasks were kept on the roof of the Department of Biological Sciences situated so that they were not exposed to direct sunlight. The temperature of the culture media ranged from 5 to 10°C. The cultures were mixed by vigorous aeration with either air (33 Pa CO_2) or high CO_2 (5·0 kPa CO_2) at a flow rate of 0·1 dm^{-3} min^{-1}.

Comparison of the photosynthetic physiology of CO_2 and HCO^-_3 utilization using low and high pH respectively assumes that the difference in pH does not adversely affect the alga (Raven, 1970). Similarly, culturing a macroalga at 5 kPa CO_2 with the consequent change in the pH of the seawater (from pH 8·0 to ph 6·15) it is assumed that there is no change in the general physiological state of the alga to the more acidic environment.

A closed IRGA ADC Type 225 Mk3 system (Analytical Development Company, Hoddeson, Herts.) was used to measure the rate of CO_2 uptake and the CO_2 compensation point of *Fucus serratus* in air as previously described (Johnston & Raven, 1986*b*). The CO_2 compensation point is defined as the concentration of CO_2 that a photosynthesizing plant can achieve in a closed system when the rate of CO_2 uptake is equal to CO_2 release. Typical values for C_3 plants are 5 Pa, and for C_4 plants 0·5 Pa. The volume of the system was 90 cm^3 and 130 to 360 mg fwt. plant material was used. The light incident on the outside of the plant chamber was 500 μmol photons m^{-2} s^{-1}. Preliminary experiments had shown that 500 μmol photons m^{-2} s^{-1} were saturating for emersed photosynthesis and did not cause photoinhibition. The plant chamber was kept at a constant temperature of 10°C with a circulating water jacket. The relationship between the concentration of CO_2 and the apparent rate of CO_2 assimilation was defined by the maximum assimilation rate (V_{max}) and the concentration of CO_2 at which the assimilation rate is half the V_{max} ($K_{0·5}$). Values of V_{max} and $K_{0·5}$ were obtained from the data using the non-linear regression program PEST (Weyers, Paterson & A'Brook, 1987).

The effect of different photon flux densities on the apparent rate of oxygen evolution by *Fucus serratus* cultured in high and low CO_2 environments was determined with a Rank oxygen electrode (Rank Bros., Cambridge). The rate of oxygen evolution was measured in seawater buffered with 25 mol m^{-3} Tris (pH 8·0) or 25 mol m^{-3} MES (pH 5·5) as appropriate with a dissolved inorganic carbon (DIC) concentration of 2·0 mol m^{-3} at both pH's. The temperature was 9°C and 100 mg fwt. of plant material was used. The light incident on the outside of the oxygen electrode chamber from a Prestinox 150W slide projector was varied using neutral density slides.

The rate of light independent carbon fixation was determined with ^{14}C and the acid-stable products were extracted and analysed using the perchloric acid/hydrogen peroxide solubilization method of Lobban (1974). Plant material was pretreated by being kept in the dark for 30 mins in 30 cm^3 seawater buffered with 25 mmol m^{-3} Tris (pH 8·0). The seawater was then labelled with 10 mm^3 stock $NaHCO_3$ [50 to 60 Ci mol^{-1} DIC (Amersham Radiochemicals, Amersham, England)] and incubated for a further 30 mins at 10°C. The plants were then rinsed in cold seawater three times, placed in liquid nitrogen and when completely frozen were weighed. The frozen plants were diced with a razor. The diced plant material was placed in a scintillation vial. 0·5 cm^3 $HClO_4$ was added to the diced material, mixed and 1·0 cm^3 H_2O_2 added. The capped scintillation vial was heated to 70°C for 2 to 3 hours or until the material had been solubilized and the solution was colourless. The vials were opened and left overnight in the fume cupboard. After the addition of 5 cm^3 Ecoscint A the radioactivity was determined with a Hewlett-Packard Scintillation Counter, 4000 Series. Preliminary experiments have shown that this method gives the same results as the more common method of hot 80% ethanol extraction and is much more convenient.

For chlorophyll *a* determinations 120 mg fwt of plant material was diced with a razor blade under low light, placed in a mortar and pestle with quartz sand and gently disrupted. Aliquots of 4 cm^3 90% acetone were added to extract the pigments and then transferred to a centrifuge tube which was kept in the dark. When the remaining material was colourless the bulked extract was centrifuged, the pellet was washed with 90% acetone, the extracts were bulked together and made up to a volume of 25 cm^3 with 90% acetone.

All glassware had been previously chilled in a refrigerator overnight and the 90% acetone kept on ice for one hour. The chlorophyll *a* concentration was calculated using the spectrophotometric equations of Jeffery & Humphrey (1975).

Many photosynthesizing plants can increase the pH of their bathing media when the exchange between atmospheric and aquatic CO_2 is limited. The final pH is termed the pH compensation point and is used as an indicator of the plant's ability to utilize bicarbonate ions (Raven, 1970; Johnston & Raven, 1986*a*), a high final pH being indicative of such a capacity. To determine whether culturing *Fucus serratus* in a high CO_2 environment affects its ability to assimilate inorganic carbon, pH-drift experiments were conducted. Plants (0·3 to 0·4 g fresh weight) were placed in test tubes with 15 cm^3 filtered seawater previously aerated overnight, suspended in a waterbath (10°C) and illuminated with four fluorescent tubes supplying a photon flux density of 120 μmol m^{-2} s^{-1}. At intervals of 3 h the pH of the seawater was measured with a Russell Combination gel filled pH electrode CE7L (Russell pH Ltd., Auchermuchty, Fife) until there was no further increase in the pH. Test tubes containing only 15 cm^3 seawater were used as controls.

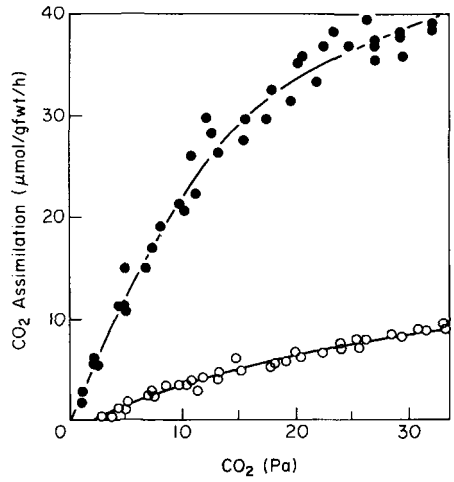

FIG. 1. Photosynthetic CO_2 assimilation as a function of CO_2 concentration for emerged *Fucus serratus* cultured at 33 Pa CO_2 (●) and 5 kPa CO_2 (○). The curves are those predicted by the estimated parameter values of V_{max} and $K_{0.5}$ given in Table I. Analysis was based on 4 individual plants for each treatment. The temperature was 10°C and the photon flux density was 500 μmol m^{-2} s^{-1}.

RESULTS

The effect on the assimilation of CO_2 of culturing *F. serratus* in high and low CO_2

When *Fucus serratus* was cultured in a high CO_2 environment the rate of CO_2 assimilation for the emersed alga at the air equilibrium CO_2 concentration (33 Pa) was below the rate for plants cultured in a low CO_2 environment (Fig. 1). The rates of CO_2 assimilation at 33 Pa CO_2 were 37·79 and 9·22 μmol CO_2 g fwt^{-1} h^{-1} for air and high CO_2 cultured algae respectively. The parameters which describe the CO_2 uptake curves for the emersed alga are given in Table I. The values for V_{max} are similar (59·39 and 48·34 μmol CO_2 g fwt^{-1} h^{-1} for high and air CO_2 cultured algae respectively) whereas the $K_{0.5}$ value is much greater from high CO_2 cultured algae (134·3 mmol m^{-3} CO_2 compared to 4·96 mmol m^{-3} CO_2). CO_2 compensation points showed a comparable change when the macroalga was cultured under high CO_2, the value increasing from 0·5 Pa CO_2 (0·049 to 0·051, 95% confidence

TABLE I. Photosynthetic characteristics of *Fucus serratus* cultured on air and 5 kPa CO_2. Numbers in brackets represent 95% confidence limits from the mean, n = (4 to 6)

	Air cultured	5 kPa CO_2 cultured
Emersed CO_2 assimilation μmol gfwt^{-1} h^{-1} (33 Pa CO_2)	37·74 (3·46)	9·22 (0·25)
Submersed O_2 evolution pH 8·0 μmol gfwt^{-1} h^{-1} (2 mol m^{-3} DIC)	31·63 (8·50)	16·89 (5·57)
Submersed O_2 evolution pH 5·5 μmol gfwt^{-1} h^{-1} (2 mol m^{-3} DIC)	42·84 (8·32)	32·62 (5·51)
CO_2 compensation point (Pa)	0·5 (0·01)	2·5 (0·2)
V_{max} of emersed CO_2 fixation μmol gfwt^{-1} h^{-1}	48·34 (4·45)	59·39 (213·69)
$K_{0.5}$ of emersed CO_2 fixation mmol m^{-3} CO_2	4·96 (0·83)	134·3 (6·54)
Submersed dark ^{14}C–DIC fixation nmol gfwt^{-1} h^{-1}	568 (45)	457 (32)
Chlorophyll *a* μg gfwt^{-1}	618 (45)	654 (49)
pH compensation point	9·10	8·11

limits) to 2·5 Pa CO_2 [2·3 to 2·7, 95% confidence limits (Table I)]. To analyse the curves of CO_2 assimilation the CO_2 compensation concentration was subtracted from each value so that a closer estimate of Michaelis-Menten parameters could be obtained. The PEST program calculates a value for the assimilation rate when the substrate concentration is zero (V_{min}) as the curve of response against log substrate concentration is sigmoidal. On this basis the V_{min} value of the PEST program was set at zero before the analysis was executed. The Hill coefficient, p, indicated that the response curve of immersed algae cultured under low CO_2 concentration closely resembled Michaelis-Menten kinetics, the value of p being near to 1.0 (Weyers, Paterson & A'Brook, 1987). For algae cultured with a high CO_2 concentration the fit was less good ($p = 0.752$).

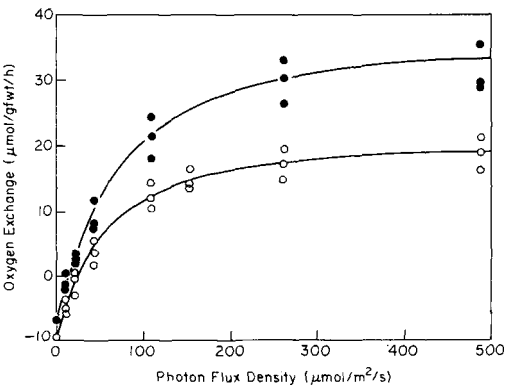

FIG. 2. Photosynthetic O_2 evolution as a function of light incident on the outside of the oxygen electrode chamber for emerged *Fucus serratus* cultured at 33 Pa CO_2 (●) and 5 kPa CO_2 (○). The curves are those predicted by the estimated parameter values of V_{max} gfwt^{-1} h^{-1}, V_{min} −6·54 μmol gfwt^{-1} h^{-1} and $K_{0·5}$ 65·39 μmol photons m^{-2} s^{-1} for 33 Pa CO_2 cultured algae and V_{max} 19·14 μmol gfwt^{-1} h^{-1}, V_{min} −8·67 μmol gfwt^{-1} h^{-1} and $K_{0·5}$ 53·03 μmol photons m^{-2} s^{-1} for 5 kPa CO_2 cultures algae estimated by the non-linear regression program PEST. Analysis was based on three individual plants for each treatment. The temperature was 10°C, the seawater was buffered at pH 8·0 with 25 mmol m^{-3} Tris and contained 2·0 mol m^{-3} DIC.

The effect on the light saturation curves of culturing *F. serratus* on high and low CO_2

The light saturated rate of oxygen evolution by the submersed alga was reduced when the alga was cultured at high CO_2 partial pressures, with a rate 16·89 μmol h^{-1} g fwt.$^{-1}$ for high-CO_2 cultured algae compared to 31·63 μmol h^{-1} g fwt.$^{-1}$ for the low CO_2 cultured algae (Fig. 2). The light compensation point (I_c) increased from 7 μmol incident photons m^{-2} s^{-1} for low CO_2 cultured algae to 28·0 μmol incident photons m^{-2} s^{-1} for high CO_2 cultured algae. The same response was observed when measurements were made at pH 5·5 (when CO_2 is the major species of inorganic carbon). The ratio of the rate of oxygen evolution from the submersed alga of cultured at high and at low CO_2 was smaller when measured at pH 5·5 (0·761) than when measured at pH 8·0 (0·534) (Table I). The light saturated rate at 2·0 mol m^{-3} was greater at pH 5·5 than at pH 8·0. Photosynthesis of algae from both treatments was saturated at about 300 μmol m^{-2} s^{-1}. The intersection (I_k) of the initial slope (α) with the photosynthetic capacity had a value of 100 μmol m^{-2} s^{-1}. The initial slope (less than 45 μmol m^{-2} s^{-1}) is used as a measure of efficiency with which the plant can utilize light to fix CO_2 at low photon flux density. Algae cultured under 5 kPa CO_2 exhibited an initial slope of only 0·058 mol O_2 mol incident photon^{-1} compared with 0·081 mol O_2 mol incident photon^{-1} from algae grown under low CO_2 (Fig. 2).

The effect of high CO_2 on levels of chlorophyll *a*, activities of dark fixation and pH compensation points

Increasing the CO_2 availability to *Fucus serratus* does not appear to have affected the levels of chlorophyll *a* or the rates of dark inorganic carbon fixation but it greatly reduced the pH compensation point (Table I). The pH compensation point at 10°C after 27 h for high CO_2 cultured algae

was 8·11 whereas the air cultured algae increased the pH to 9·10.

DISCUSSION

Our current work on inorganic carbon concentrating mechanisms had been restricted to *Ascophyllum nodosum* (L.) Le Jolis which is the main local intertidal macroalga. Due to the difficulty of reproducing in culture the continuous emersion/immersion cycle, *Fucus serratus* was selected for this study as an intertidal macroalga with a C_4-type physiology (Surif & Raven, 1989) which was likely to be able to tolerate a three week period of continual immersion as it inhibits the lower intertidal zone. Thus it is important to show that the continual immersion did not greatly affect its photosynthetic physiology. The high rates of CO_2 assimilation (in air) and oxygen evolution (in water), the low CO_2 compensation point (in air), the high pH compensation point (in water), the levels of chlorophyll *a* and the high affinity for CO_2 compare favourably with values found in the literature (Seybold & Egle, 1938; King & Schramm, 1976; Küppers & Kremer, 1978; Surif & Raven, 1989). The rates of dark fixation are about 0·8 of those previously reported (Küppers & Kremer, 1978) assuming a wet weight: dry weight ratio of 0·246 (Seybold & Egle, 1938) though this difference may be due to seasonal differences (Johnston & Raven, in prep.). Dark fixation was only 1.5% of the photosynthetic rate and this is likely to be a maximum value as the photosynthetic rate was based on the whole plant whereas the dark fixation rate was obtained from the apical tissue (see Küppers & Kremer, 1978 for longitudinal variation of photosynthesis and dark fixation in *F. serratus*).

The $K_{0.5}$ CO_2 for *Fucus serratus* cultured under low CO_2 of 4·96 mmol m^{-3} is less than the previously reported value of 15·50 mmol m^{-3} CO_2 for the intertidal macroalga *Ascophyllum nodosum* (Johnston & Raven, 1986*b*). The apparent $K_{0.5}$ for CO_2 is also less than the reported K_m CO_2 values for ribulose bis-phosphate carboxylase/oxygenase [RUBISCO (see Kerby & Raven, 1985; Davison, 1987)] and thus supports the suggestion that an inorganic carbon concentrating mechanism is operating in *F. serratus* (Kerby & Raven, 1985).

There have been no previous reports on the effect of elevated CO_2 concentrations on marine macroalgae. Culturing *Fucus serratus* under 5 kPa CO_2 for three weeks had a considerable effect on its photosynthetic physiology. The rate of CO_2 assimilation in air was greatly reduced; the rate of oxygen evolution in water decreased to a lesser degree. The CO_2 compensation point increased and there was a shift in the light saturation curve with an increase in the light compensation point and decrease in the initial slope. The observed pH compensation values reported here may not be maximal values; Surif & Raven (1989) reported a pH compensation point of 9·725 for *F. serratus*, and our lower value is likely to be due to sub-saturating light levels but culturing in high CO_2 has greatly reduced this indicator. The lower pH compensation point, higher CO_2 compensation point and $K_{0.5}$ of CO_2 uptake all indicate that the inorganic carbon concentration mechanism has been suppressed. As can be seen from Table I, the 95% confidence limits for the V_{max} of algae cultured under high CO_2 is greater than the V_{max} value itself. This is because the range of CO_2 concentrations over which assimilation could be analysed was restricted to the substrate dependent portion of the uptake curve. That the affinity for CO_2 declined following culture in high CO_2 can be seen from the greater decrease in CO_2 assimilation in air than in 2 mol m^{-3} DIC seawater. The assumption that the prolonged period of submersion in high CO_2 and the consequent lower than normal pH did not adversely affect the overall physiological state of the plants is central to the study of CO_2/HCO^-_3 utilization. The lack of any change in the levels of chlorophyll *a* and dark fixation rates after the algae had been cultured in high CO_2 suggests that the low pH did not adversely affect the algae. Further work is necessary to establish whether the effect of the high CO_2 environ-

ment on macroalgae is due to the high concentration of CO_2 or the low pH.

Assuming that the equal levels of chlorophyll *a* in low and high CO_2 cultured algae mean equal absorptances, then the higher α from algae cultured in low CO_2 (Fig. 2) suggests that these algae are more energy efficient than those cultured in high CO_2. In terms of absolute values of α, the value of 0·081 mol O_2 mol incident photon^{-1} presented in this paper is close to that reported for *Fucus serratus* by Lüning & Dring (1985) of 0·07 mol O_2 photon^{-1} for low CO_2 cultured algae. Our value of α based on incident photons would be expected to be lower than their value of α based on absorbed photons, though valid comparisons between these two values are made difficult by differences in equipment, light sources and geometry.

As the rate of dark fixation did not change significantly between treatments and the K_m for HCO^-_3 of phosphoenolpyruvate carboxykinase is so large (1·084 mol m^{-1} CO_2, Johnston & Raven, 1989) it seems unlikely that β-carboxylation is directly involved in the low apparent $K_{0·5}$ for CO_2 assimilation. From the other parameters which are affected by the high CO_2 environment (photon yield, pH compensation point, CO_2 compensation point and DIC affinity), it is possible to distinguish between repression of active DIC entry (either CO_2 or $CO_2+HCO^-_3$), repression of carbonic anhydrase (CA) or repression of both as the cause of the responses to culture at high CO_2. The reported control by temperature of high and low photorespiratory states for some freshwater macrophytes (Salvucci & Bowes, 1981) may be related to a high/low CO_2 adaptation. With the same concentration of RUBISCO, a plant growing at a higher temperature and in higher light will have a greater biochemical potential for fixation but a less favourable O_2/CO_2 ratio at the site of RUBISCO (Ku & Edwards, 1977) which would result in a high photorespiratory state.

At a low pH, a higher rate of oxygen evolution than that observed at a higher pH with the same DIC concentration is often used to indicate that photosynthetic cells have a greater affinity for CO_2 than for HCO^-_3 ions. The decrease of inorganic carbon saturated oxygen evolution at pH 5·5 in plants cultured in high CO_2 indicates that the capacity for carboxylation is reduced. The rate of oxygen evolution at pH 8·0 (5·78 μmol m^{-2} s^{-1}, using a conversion factor from mol g fwt^{-1} h^{-1} to mol m^{-2} s^{-1} of 5·407, Johnston, 1984) is still greater than the theoretical rate at which CO_2 could either diffuse through an unstirred layer (0·405 μmol m^{-2} s^{-1}, unstirred layer assumed to be 50 μm thick with the CO_2 gradient 15·01 mmol m^{-3}) or be produced in the unstirred layer from the dehydration of HCO^-_3 to CO_2 (Johnston & Raven, 1986*a*; Cook, Lanaras & Colman, 1986; Surif & Raven, 1989). *Fucus serratus* cultured under high CO_2 for three weeks still has the ability to utilize bicarbonate ions but the lower pH compensation point and reduced rate of oxygen evolution at pH 8·0 compared to the rate of pH 5·5 suggests that the alga is no longer able to do so to the same degree as air cultured algae.

To obtain a better understanding of the process of acclimation to a high CO_2 environment further work is required to ascertain whether the shift from the C_4-type gas exchange physiology to the C_3-type is associated with a change in the levels of carbonic anhydrase (Colman *et al.*, 1985, Miyachi Tsuzuki & Yagama, 1985).

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